



Low seed viability and germination in *Polylepis flavipila* hinder forest restoration: The role of seed mass and maternal effects

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ABSTRACT

Seed physiological quality is usually determined by the interaction between genetics and the mother tree's environment, as well as by intrinsic characteristics, such as seed mass. In the Andean highlands, massive sapling production for forest restoration projects is constrained by seed availability and quality. There, species of the genus *Polylepis* dominate the remnant forests. The associations of seed viability with seed mass and maternal effects were evaluated in *P. flavipila*, a threatened tree species endemic to the Peruvian Andes. The characteristics and environments of 18 mother trees from two sites were measured, and seed quality was assessed in three tests. Seed mass was 3.49 ± 1.76 mg (range: 0.5–16 mg) and showed the greatest variability within mother trees, followed by variability among trees and among sites. Viability rates, standard germination and greenhouse germination (2.06 ± 1.35 %, 0.59 ± 0.89 % and 0.64 ± 1.11 %; respectively) were low at both sites. These results were attributed to the presence of seeds with non-viable or absent embryos. Seed viability increased with seed mass and mother tree height, and was positively associated with number of seedlings ($r = 0.56$). The deficient seed viability and germination found in *P. flavipila* are the lowest reported for the genus. The low seed physiological quality detected is a risk factor that exacerbates the species' degree of threat, posing a challenge for sapling production. The reported associations of *P. flavipila* seed viability with seed mass and maternal characteristics and environment may guide the selection of better seed quality and serve as a basis for future studies on the challenges and limitations of the reproductive biology of this species.

1. Introduction

Restoring an ecosystem entails recovering its structure, functionality, and sustainability after it has been degradation or destroyed (Gann et al., 2019; SER, 2004). However, the implementation of ecological restoration faces various bottlenecks, including adequate seed supply chains (Cross et al., 2020; Jalonon et al., 2018; León-Lobos et al., 2020). Seed availability and quality can determine the success or failure of

many restoration projects (Broadhurst et al., 2015, 2008; Gann et al., 2019), particularly when the objectives are large-scale or funding is limited (Merritt and Dixon, 2011). It is therefore crucial to adopt effective and predictable seed-based restoration practices (INSR, 2024; Larson et al., 2023; Merritt et al., 2016).

Seed quality is assessed primarily at the physical (purity) and physiological (viability and germination) levels, for both native (Cross et al., 2020) and commercial seeds (ISTA, 2025). Seed physical quality

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depends on collection, selection, processing, and cleaning (Pedrini and Dixon, 2020), while seed physiological quality depends primarily on the genotypic and phenotypic factors affecting the mother tree during seed development. These factors, known as "maternal effects", can be related to the maternal environment or can manifest through the maternal characteristics of the tree, directly affecting seed viability and germination (Baskin and Baskin, 2019; Galloway, 2005; Guterman, 2000; Penfield and MacGregor, 2017).

The maternal environment encompasses a variety of micro- and mesoscale factors influencing tree development and reproduction. Among these, climatic variables such as temperature (Dewan et al., 2018; Dwyer and Erickson, 2016; Penfield and MacGregor, 2017) have been shown to influence seed quality. Likewise, in natural environments, altitude (Carón et al., 2018; Marcora et al., 2008) has been reported to affect seed quality, probably as an indicator of the effect of temperature. Similarly, the number of daylight hours (Cheplick and Sung, 1998; Galloway, 2005; Luzuriaga et al., 2006), soil moisture and nutrients (Baskin and Baskin, 1998; Cheplick and Sung, 1998; Luzuriaga et al., 2006) were found to condition the resources available for the formation of fruits and seeds. In addition, the slope of the land where the tree is established influences soil humidity, nutrient content and texture as well as the amount of light received, with the consequent effect on tree development (Li et al., 2023; Morbidelli et al., 2018; Wang et al., 2023). Furthermore, habitat fragmentation and degradation (Ashworth and Martí, 2011; Renison et al., 2004) reduce the fitness of the maternal tree, generating both intra- and interspecific variations in seed viability and germination.

Maternal characteristics also influence seed physiological quality. Tree height (Domic et al., 2020; Goszka and Snell, 2020), diameter (Viglas et al., 2013) and age (Alonso-Crespo et al., 2020; Bisht et al., 2016; Viglas et al., 2013) were found to positively influence percentages of seed germination or viability. The positive relationship between tree size and seed physiological quality may be attributed to the fact that large trees have easier access to water and nutrients and a greater capacity to mobilize and manage those resources than small trees.

In addition, some factors associated with seed physiological quality, such as mass, size, or volume, are specific to each seed. Previous studies have shown that larger seeds have higher viability (Domic et al., 2020; Du and Huang, 2008; Galíndez et al., 2009; Goszka and Snell, 2020; Leishman et al., 2000; Seltmann et al., 2007; Suárez-Vidal et al., 2017). Indeed, larger seeds contain greater nutrient and water reserves, which are essential for the initial embryo and seedling development. In addition, they are better adapted to adverse conditions due to their high energy storage capacity and robust physical structure (Fenner and Thompson, 2005; Moles and Westoby, 2004; Westoby et al., 1996). However, these facts often do not apply to *Polylepis*, since many of its seeds, even heavy ones, are non-viable (Domic et al., 2020; Seltmann et al., 2007; Vega et al., 2018).

The genus *Polylepis* includes 47 species of trees and shrubs distributed along the Andes and the 'Sierras de Córdoba' in Argentina (Boza Espinoza and Kessler, 2022; Gamarra and Valdivia, 2024; Quispe-Melgar et al., 2024b). These species usually dominate high mountain ecosystems range between 900 and 5000 m a.s.l.; therefore, they are of great importance in the ecological and sociocultural balance of the Andes mountain range (Fjeldså and Kessler, 1996; Kessler, 2006). However, centuries or millennia of human impact have significantly fragmented and reduced these forests, placing them among the most threatened ecosystems worldwide (Fjeldså and Kessler, 1996; Kessler, 2006). Natural regeneration of *Polylepis* includes key aspects, such as seed production, germination, seedlings establishment and sapling survival. *Polylepis* produces seeds that are dispersed by gravity (i.e. barochory) up to 6 m around the tree (Torres et al., 2008). In this sense, Enrico et al. (2004) reported that the main "filter" affecting the regeneration of *P. australis* forests occurs during seed germination, which is closely related to seed quality. Thus, trees that produce a higher proportion of viable seeds are likely to be surrounded by a greater number

of seedlings than trees that produce mainly non-viable seeds. However, factors other than seed viability also affect regeneration, including the availability of suitable microsites for germination, high soil erosion, and stressful environmental conditions that hinder seedling establishment and survival (Cáceres et al., 2019; Pollice et al., 2013; Torres et al., 2008).

Our general objective was to evaluate the associations of seed mass, maternal characteristics and environment with *P. flavipila* seed viability. To this end, we: (1) evaluated whether seed mass varies among mother trees and between sites; (2) examined the relationship between seed mass and seed viability, controlling for the effects of sites and maternal trees; (3) evaluated the influence of maternal characteristics and the maternal environment on seed viability; and (4) investigated whether seed viability values were correlated with germination rates recorded in both standard and greenhouse settings.

We hypothesize that (H₁) seed mass would be positively associated with seed viability and germination because embryonic development is greater in heavy seeds than in light seeds; (H₂) maternal characteristics and the maternal environment would influence seed physiological quality, since the size of the tree and its immediate environment affect its access to water, light, and nutrients. These effects are expected to be enhanced by a favorable maternal environment; and (H₃) seed viability or germination would be positively associated with the number of seedlings under the crown of the mother tree.

2. Materials and methods

2.1. Study species

Polylepis flavipila (Bitter) M. Kessler & Schmidt-Leb (2006) is one of the 17 endemic species reported for Peru. Its distribution is restricted to the western slopes of the central Andes (Lima, Huancavelica and Ayacucho), occupying dry and cold areas between 3300 and 4660 m a.s.l. (Boza Espinoza and Kessler, 2022). Large populations are found in the Nor Yauyos Cochabamba Landscape Reserve, an area of high biodiversity (Quispe-Melgar et al., 2020; Trinidad and Cano, 2016), but that undergoes human disturbances such as fires, livestock grazing, firewood extraction, agriculture and road construction, along with infestation by the hemiparasite *Tristerix chodatianus* (Camel et al., 2019a). The cover of *P. flavipila* has been fragmented and reduced by 53 % in the last 45 years (Ames-Martínez et al., 2021). For this reason, it is classified as Vulnerable in the Red Book of Endemic Plants of Peru (Mendoza et al., 2006) and Endangered by the IUCN (Mendoza and Boza Espinoza, 2024).

Polylepis flavipila is a small tree or shrub, reaching 3 to 8 m in height, with population densities of 390 to 1135 stems/ha; most stems are <25 cm in diameter. Soil organic matter, nitrogen, and water availability influence forest structure, while human activity negatively affects forest dasometric characteristics (Arzapana-Almonacid et al., 2022; Camel et al., 2019b). Reproduction is primarily sexual, with very few asexual clones present (personal observation) and no documented phenology. Fruits are turbinate and serve as dispersal units (hereafter referred to as "seeds"). Seeds have not been reported as food for wildlife, although livestock may consume them while browsing the stems. Infestation by *T. chodatianus* affects tall trees (probably those with great reproductive potential), reducing flower and seed production, and negatively impacting reproductive success (Arzapana-Almonacid et al., 2022; Camel et al., 2019a).

2.2. Study area

We selected two sites that were at a 75-km distance along the western mountain range of the central Peruvian Andes: (1) Manta (12°37'20"S, 75°09'51"W, 4220–4650 m a.s.l.) and (2) Castrovirreyra (13°17'27"S, 75°17'38"W, 4200–4480 m a.s.l.). The landscapes in these sites are dominated by relict *P. flavipila* forests, surrounded by a matrix of shrublands, grasses, rocky outcrops, and bare soil (Fig. 1A-C).

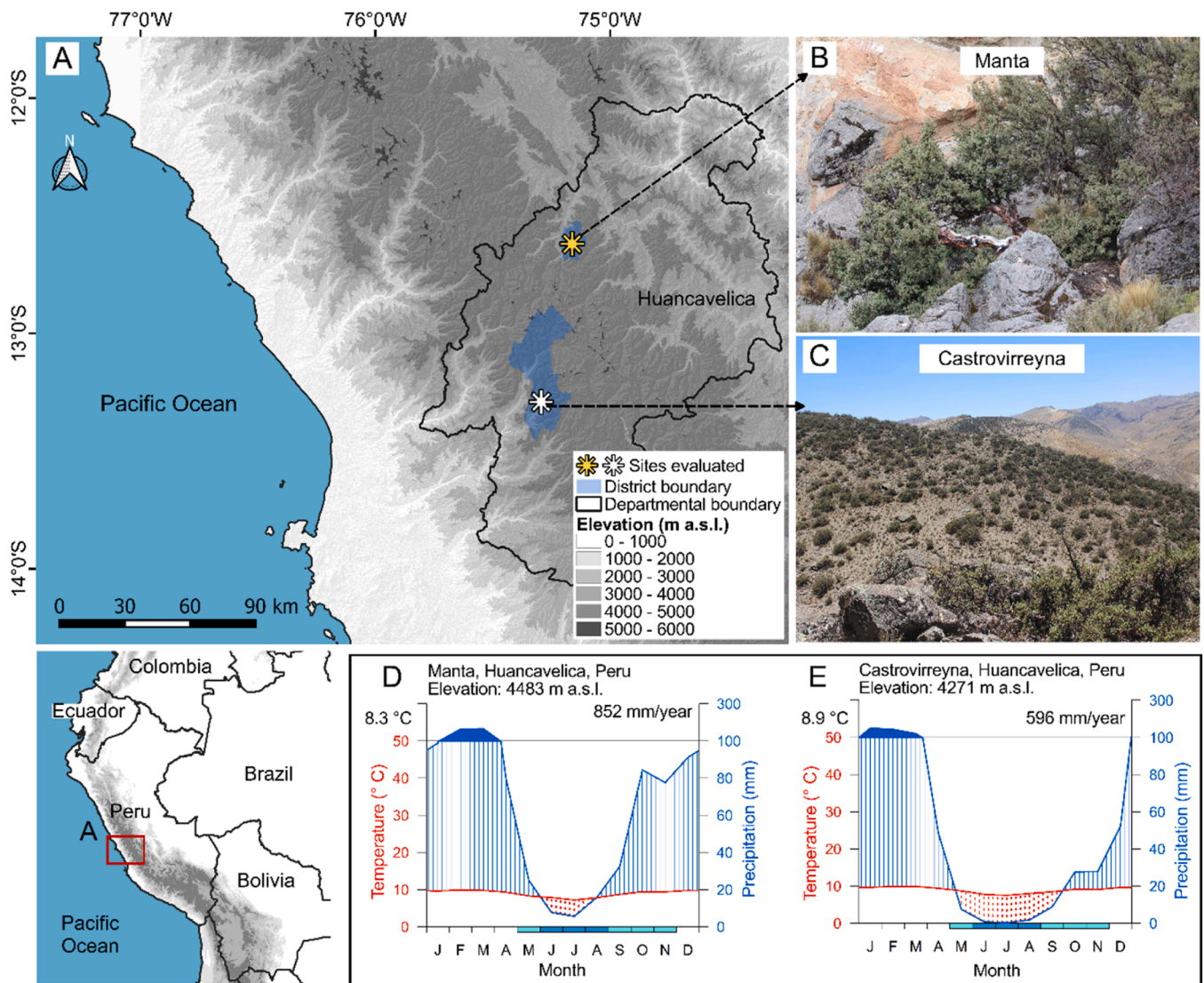


Fig. 1. . A Location of the study area. B–D Study sites of *Polylepis flavipila*. C–E Walter and Lieth climate diagram for each study site (mean of 35 years; 1981–2016). The diagram shows mean temperature and precipitation values; a period with precipitation (blue line) above and below 100 mm is considered “wet” (solid blue area) and “humid” (vertical blue lines), respectively. When the precipitation line (blue line) is lower than the temperature line (red line), the period is considered “dry” (area with red dots). The months of “probable” and “definite” frosts are indicated with blue and light blue boxes, respectively, at the bottom of the diagram.

Additionally, they are impacted by livestock farming, burning, and logging. Mean temperature is 8.6 ± 7.22 °C (minimum = 1.87 ± 2.3 °C, maximum = 15.9 ± 0.6 °C), with the lowest temperatures being recorded in June and August. Mean annual precipitation is 723.63 ± 55 mm, concentrated between October and April (>90 %), with July being the driest month (Fig. 1D & E). Both sites belong to the high-Andean Relict Forest and Humid Puna Grassland ecosystems; however, annual precipitation is higher in Manta (MINAM, 2019) (Fig. 1D & E). The climatic data are means of the daily data obtained from the Grillado PISCO v2.1 product – SENAMHI, for the period 1981–2016 (Aybar Camacho et al., 2017; Huerta et al., 2018).

2.3. Field sampling

In February and March 2021, we established linear transects in each evaluated forest. We selected 18 trees—9 from each site. The trees were located within 25 m of river margins, which we used to define the midline of the transect and which facilitated our access to the forests. The trees extended from 4220 to 4650 m a.s.l. in Manta site and from 4200 to 4350 m a.s.l. in Castrovirreyna site. Trees were selected for their

vigorous appearance, the presence of fruits, and the lack of visible damage from fungi, insects, hemiparasites, or other factors (Pérez-Harguindeguy et al., 2013).

2.4. Maternal characteristics and environment

We measured the following maternal characteristics in the selected trees: (1) height, (2) stem diameter at ground level (calculated by measuring the stem circumference) and (3) crown diameter (mean of two diameters measured in east-west and north-south orientations). We also counted (4) the number of seedlings established in an area defined by a 6-m radius circle centered at the base of the tree. This area corresponds to the maximum seed dispersal distance reported for *P. australis* (Torres et al., 2008). *Polylepis flavipila* seedlings were defined as individuals of up to 15 cm in height (Enrico et al., 2004).

We additionally recorded data from the mother tree environment: (1) elevation above sea level (m a.s.l.), (2) slope (%) and (3) rock cover under the tree crown (%) (Marcora et al., 2008; Torres et al., 2008).

2.5. Seed collection

In the field, mature seeds were manually collected and kept in separate bags corresponding to each mother tree. Mature seeds were identified by their easy detachment from the cluster and their light brown color (Gutiérrez et al., 2015). The quantity of seeds collected varied with the productivity of the tree and the access to the tree crown. In the laboratory, the seeds were dried at room temperature without receiving antifungal treatment. The total mass (TM) of seeds collected from the 18 trees was 94.38 g (24.02 g from Manta and 70.36 g from Castrovirreyna). Subsequently, we analyzed the purity according to ISTA (2025). For this, impurities such as hollow or broken seeds (damage > 50 %) and plant or inert material were excluded; we obtained 30.31 g of Pure Seed (PS), or Pure Seed Units (PSU) according to Pedrini and Dixon (2020). The collected seed lot had a purity of 32.4 % (% Purity = (PS / TM) × 100).

2.6. Seed number and mass

To evaluate the physiological quality of the seeds, the Pure Seed (PS) sample, composed of 8680 seeds (482.22 ± 257.99 seeds per tree), was divided using the hand halving method (ISTA 2025). This resulted in three subsamples (S1, S2 and S3), which were randomly assigned to viability, standard germination, and greenhouse germination tests. The mass (mean ± SD) of seeds in S1, S2 and S3 was 3.54 ± 1.75 mg, 3.45 ± 1.72 mg and 3.48 ± 1.83 mg, respectively, without significant differences (ANOVA, $F_{[2, 8677]} = 1.8, p > 0.05$). The number of seeds in the three subsamples was similar (2867, 2934 and 2879 seeds in S1, S2, and S3, respectively), which demonstrates the homogeneity of the subsamples. Seeds were labeled according to their mass, mother tree, and site; the same information was used in all labels in all analyses. Then, they were stored at 5 °C for two months until the start of the tests.

2.7. Seed viability

We evaluated seed viability using the Tetrazolium topographic test (ISTA, 2025) on S3 (2879 seeds in total/159.94 ± 89.89 seeds/tree on average). Our focus was on the individual behavior of each seed rather than the mean per repetition. Seeds were immersed in water at 15 °C for 24 h, then cut longitudinally and exposed to a 1 % solution of 2,3,5-triphenyltetrazolium for 12–18 h. Viability was assessed by examining the presence and/or coloration of embryonic tissues, following an adaptation of ISTA (2025) and the methodology of Vega et al. (2018) (see Table 1). Each seed was assigned binary viability values (Viable = 1, Non-viable = 0) and classified into categories (C1, C2, C3, or C4). The viability rate per tree was calculated as $V(tree) = (viable\ seeds$

Table 1

Categories of seeds proposed for the tetrazolium viability test, considering the coloration and/or presence of embryonic tissues.

Category	Description
C1: Viable seed	Embryonic tissue with complete structures (cotyledons, hypocotyl-radicle axis, testa and tegmen). Embryo completely stained bright red and with a firm and compact appearance, without signs of softness or watery texture.
C2: Viable seed with a proportion of non-viable tissue	Embryonic tissue present, but with a non-viable portion. >3/4 of the embryo (cotyledons and hypocotyl-radicle axis) is stained bright red, and it has a firm appearance, without a watery or soft texture.
C3: Non-viable seed – seed with degraded or dead embryo tissue	Embryonic tissue present but degraded, with white, hyaline or brown (necrotic) coloration or a watery and soft texture. Tetrazolium staining is weak or absent, reflecting the lack of metabolic activity.
C4: Non-viable seed – seed without embryo tissue	Completely empty seed, without embryo or internal integuments (testa and tegmen).

/seeds per tree × 100) and the total viability rate was calculated as $V(total) = \Sigma(V(tree)/number\ of\ trees)$.

2.8. Standard germination

Standard germination was tested using S2, with 2934 seeds in total and 163 ± 90.02 seeds per tree, following the methodology outlined by ISTA (2025) and using the same approach as that employed for the viability test. The seeds were disinfected with 1 % NaClO and a systemic fungicide, and then arranged evenly in Petri dishes on a moist filter paper substrate. The dishes were labeled according to seed mass, mother tree, and site, and placed in an incubator set at 20 °C with 12-hour light/dark cycles and constant humidity. Seed germination was recorded every two days, with germination being determined by the emergence of the radicle (Pedrini and Dixon, 2020). The experiment ended after 40 days, when no further germination was observed. The standard germination rate per tree was calculated as follows: $SG(tree) = (germinated\ seeds/seeds\ per\ tree \times 100)$ and the total standard germination rate, as follows: $SG(total) = \Sigma(SG(tree)/number\ of\ trees)$.

2.9. Greenhouse germination

A greenhouse was equipped with a polycarbonate roof and 70 % Raschel mesh on the sides. Two germination beds (6 m long x 0.8 m wide x 0.4 m high each) were constructed and disinfected sand (10 % NaClO and boiled water) was used as substrate (ISTA, 2025). The beds were divided into 20-cm² sections, where seeds from S1 (2867 seeds in total/159.28 ± 89.80 seeds per tree on average) were sown at a depth of 1 cm, taking into account the identity of each seed (mass, mother tree, and site). Germination was recorded every two days, with germinated seeds being identified by the emergence of the hypocotyl. The experiment ended after 70 days, when no further germination was observed, and the seedlings were transplanted. The germination rate per tree in the greenhouse was calculated as follows: $GG(tree) = (germinated\ seeds/seeds\ per\ tree \times 100)$ and the total greenhouse germination rate was calculated as follows: $GG(total) = \Sigma(SN(tree)/number\ of\ trees)$.

2.10. Data analysis

Statistical analyses were performed using R software version 4.2.2 (R Core Team, 2022). Initially, we assessed whether seed mass varied among sites or among mother trees using a generalized linear model (GLM) with gamma distribution, followed by multiple comparisons of means with Tukey contrasts (Hothorn et al., 2008).

To analyze the influence of intra- and inter-individual level factors (e.g., seed mass and maternal effects, respectively) on seed physiological quality, we used a two-stage modeling approach, since it allows for the analysis of structured data and controls for non-independence and pseudoreplication. This approach has been applied in ecology (e.g., Hill et al. (2017); Karavarsamis and Huggins (2020); Yang and Huang (2015)) and other scientific fields (e.g., Sedighi-Maman and Mondello (2021); Yang et al. (2021)).

At the first stage, we assessed seed physiological quality using viability data, which had fewer zeros than data obtained from the other tests. Seed viability was modeled using generalized linear mixed models (GLMMs) with a binomial distribution (logit link function) and nested random effects. Seed mass (centered and standardized) (Schielzeth, 2010) and assessment sites were the explanatory variables with fixed effects. Site was included as a fixed effect due to the limited number of levels (two levels: Manta and Castrovirreyna) (Gelman and Hill, 2006). The tree was modeled as a random effect, nested within sites, reflecting a nested experimental design. Using the lme4 package (Bates et al., 2015), we fitted two models: one with random intercepts and slopes (Eq. 1), and one with random intercepts only. Models were compared using both the parametric bootstrap and the likelihood ratio test (Inchausti, 2022),

with 500 simulations via the pbrktest package (Halekoh and Højsgaard, 2014). We calculated the conditional and residual R^2 to measure the goodness of fit of the models (Nakagawa and Schielzeth, 2013); the interpretation of the numerical model parameters follows the “Rule of 4” of Gelman et al. (2014).

Model1 = glmer(Viability ~ mass × site + Mass|Site : Tree), family = binomial(link = logit), data)

(1)

At the second stage, we applied a multi-model inference approach (Barton, 2022; Burnham and Anderson, 2004). We transformed random intercepts and slopes to the scale of the data using the invlogit function (Gelman and Yu-Sung, 2024); we used both variables as response variables, and maternal characteristics and maternal environment as explanatory variables; we did not include number of seedlings. We generated candidate models using the MuMIn package (Barton, 2022); we did not include variables with a Pearson correlation $r \geq 0.7$ in the same model (Dormann et al., 2013). We selected the most parsimonious model using the corrected Akaike Information Criterion (AICc) (Burnham and Anderson, 2004). Finally, we calculated the Pearson correlation between the number of seedlings under the tree crown and the average seed viability rate.

We assessed the validity of the models by a visual analysis of the Dunn-Smyth residuals, which we obtained using the DHARMA package (Hartig, 2022). We evaluated the graphical analysis of the residuals versus the predicted values, the theoretical versus sample quantile distribution, the relationship between the residuals and each explanatory variable, and Cook’s distances. In these assessments, no obvious deviations from homoscedasticity or normality were detected.

3. Results

3.1. Seed mass, mother trees and sites

The seed mass (mean \pm SD) of *P. flavipila* was 3.49 ± 1.76 mg,

ranging from 0.5 to 16 mg. Most (87 %) seed masses were within ± 1 standard deviation of the mean (1.73 to 5.25 mg), and displayed a positive skew with an inverted J shape (Fig. 2A), similar to a gamma distribution (Δ AIC of 1518.32 and 463 compared to the normal and lognormal distributions).

Seed mass varied with the site of origin ($\chi^2_{[1]} = 106.89, p < 0.01$, GLM Gamma), with seeds from Castrovirreyña (3.76 ± 1.90 mg) being heavier than those from Manta (2.96 ± 1.32 mg) (Fig. 2B). Seed mass also varied significantly between mother trees, with mean values per mother tree ranging from 1.81 to 5.70 mg ($\chi^2_{[17]} = 691.09, p < 0.01$, GLM Gamma) (Fig. 2C & D). Variability was greater between mother trees than between sites (Fig. 2B-D), with maternal trees and sites explaining 29.61 % and 4.58 % of variance, respectively. However, the greatest variation occurred within trees, with mean minimum and maximum values of 1.11 and 8.56 mg, respectively (1.11 to 6.89 mg in Manta and 1.11 to 10.2 mg in Castrovirreyña).

3.2. Seed viability

Seeds of *P. flavipila* contained an embryo, consisting of cotyledons, the hypocotyl-radicle embryonic axis, and two integuments (testa and tegmen) (Fig. 3A & B). Occasionally, we observed some fruits of mass greater than 10 mg, which contained two to four seeds; however, in all

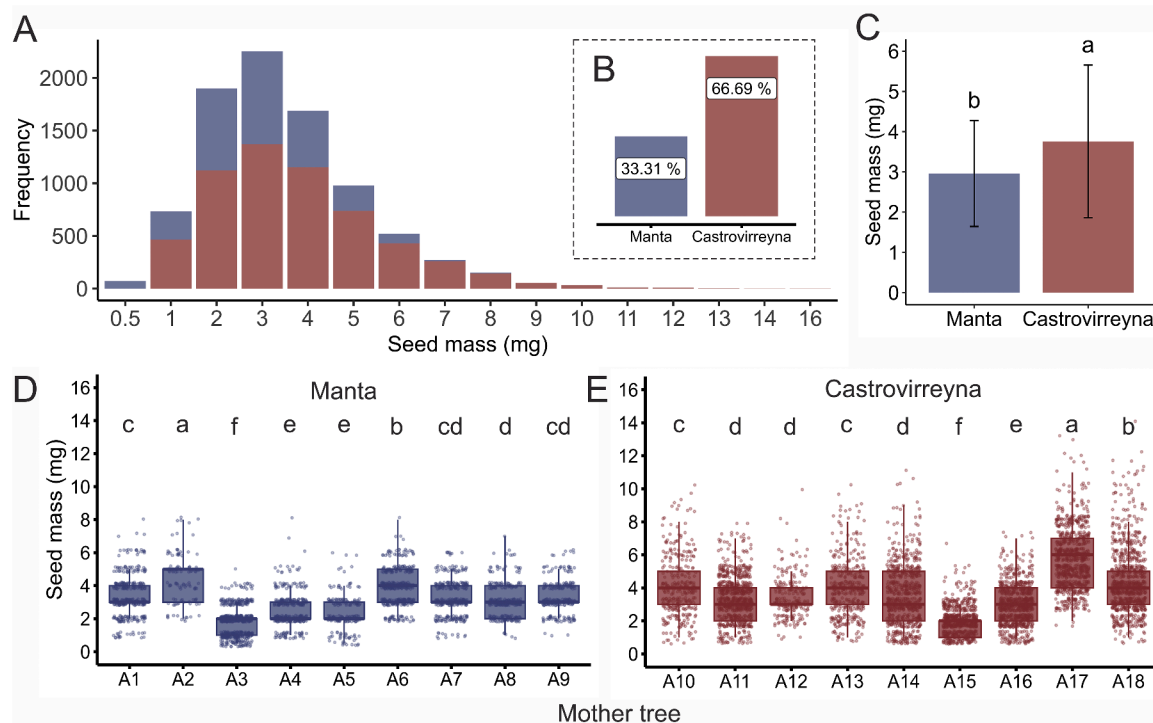


Fig. 2. A. Histogram of *Polylepis flavipila* seed mass and proportion of seeds collected from each site. B. Bar plot of seed mass in relation to the evaluated sites. C and D. Boxplot of the seed mass for each tree evaluated. The vertical lines in figure B represent ± 1 SD and the letters above the bars or box plots represent the results of the Multiple Comparisons of Means - Tukey Contrasts. Different letters indicate statistically significant differences between stages ($p < 0.05$).

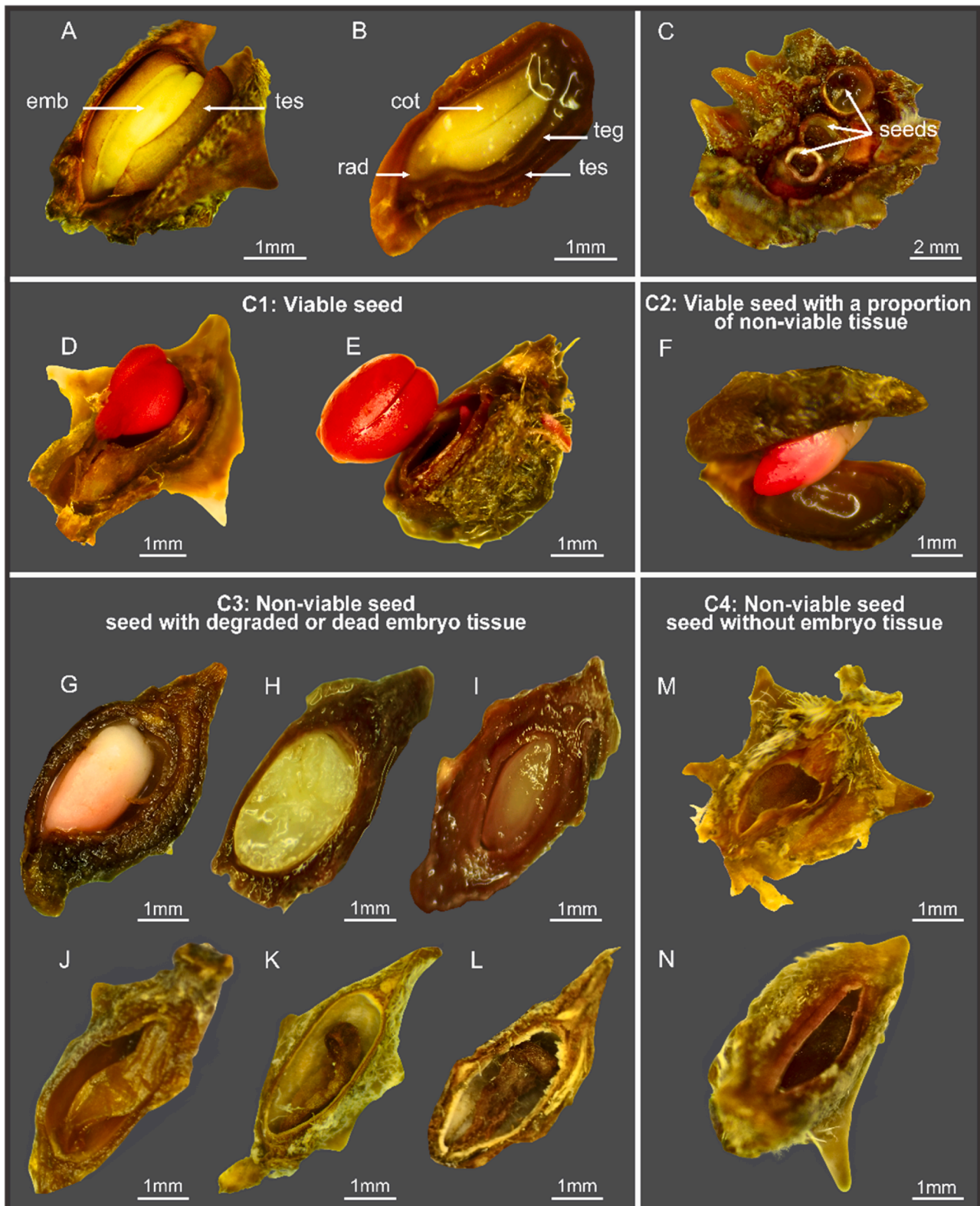


Fig. 3. *P. flavipila* seeds and categories of seed viability according to coloration and presence of embryonic tissue (embryo and integuments). A and B Details of the seeds in front and longitudinal view. C Fruits with two or more seeds present. emb: embryo; tes: testa; teg: tegmen; cot: cotyledons; rad: radicle. D and E C1: Viable seed, seed with embryo tissue completely stained bright red with normal and firm appearance. F C2: Viable seed with a proportion of non-viable tissue, seed with embryo tissue mostly stained red (>3/4 of the embryo). G-L C3: Non-viable seed – seed with degraded or dead embryo tissue. M and N C4: Non-viable seed – seed without embryo tissue, embryo, testa and tegmen absent (empty seed).

cases, these seeds had non-viable embryos (Fig. 3C).

The selected *P. flavipila* mother trees had a seed viability rate (mean \pm SD) of 2.06 ± 1.35 % (minimum = 0, maximum = 5 %). Seeds were classified into four viability categories (Fig. 3d-N). C1: Viable seed (1.29 % of seeds) with embryo tissue completely stained bright red (Fig. 3D & E). C2: Viable seed with a proportion of non-viable tissue (0.63 % of seeds): embryo tissue mostly stained red (>3/4 of the embryo) (Fig. 3F). C3: Non-viable seed with degraded or dead embryo tissue: (75.20 % of seeds) white, hyaline or brown embryo tissue in a watery or degraded state (Fig. 3G-L). C4: Non-viable seed without embryo tissue: (22.89 % of seeds) completely empty seeds (Fig. 3M & N).

In both sites, seed mass varied among viability categories ($\chi^2_{[3]} = 33.89$, $p < 0.01$), with the mass (mean \pm SD) of seeds from the viable seed categories C1 and C2 (6.14 ± 2.66 mg) being greater than the mass of the seeds in the non-viable seed categories C3 and C4 (3.43 ± 1.77 mg) (Fig. 4).

3.3. Seed mass and seed viability

Viability of seeds was positively influenced by seed mass ($\beta = 1.86$, $SE(\beta) = 0.53$, 95 % CI [0.82; 2.89], $p < 0.001$), while site and site \times mass interaction had no significant effects ($p > 0.05$) (Table 2 and Fig. 5A). According to the results of the model, seeds with a mean mass value (3.49 mg) and from the Manta site had a 1.63 % probability of being viable, and for the Castrovirreyra site, this probability was 0.56 %. However, an increase of 1 SD (1.76 mg) in seed mass is associated with a maximum increase of 46.5 % in the probability of viability in Manta site and 36 % in Castrovirreyra site. Thus, seed mass was the best predictor of viability. Moreover, the model had a marginal R^2 of 30.5 (for fixed effects) and a conditional R^2 of 56.76 (for fixed and random effects), indicating that the inclusion of random effects enhanced the model's ability to explain variability in the data, despite the large number of zeros (Table 2).

The intercept differential for the Castrovirreyra site (-1.06) had low precision (Table 2), due to the great differences in seed viability between mother trees. This variability is reflected in the wide confidence intervals of the model predictions for each site (Fig. 5C). The intercept and random slope for each evaluated tree showed notable variations associated with individual trees (Fig. 5B). Therefore, including the variations of mother tree coefficients contributed to the accuracy of the modelling of data variability (Fig. 5D).

3.4. Seed viability, maternal characteristics and maternal environment

For the random intercept, the best model included mother tree height as a statistically significant predictor variable ($p < 0.05$). For each additional meter in mother tree height, seed viability increased by 1.3 %. For the random slope, the best model was the null model, since it did not include any predictor variables (Table 3). The number of seedlings had a moderate positive association with seed viability ($r = 0.56$, $r^2 = 30.91$ %, $p < 0.05$). The results showed that for each additional meter in mother tree height, seed viability increased by 1.3 %, and that approximately 31 % of the variation in seedling number can be explained by seed viability.

3.5. Standard germination and greenhouse germination

In the standard germination test, seed germination began after 15 days and ended after 40 days, with a germination (mean \pm SD) of 0.59 ± 0.89 % (20 germinated seeds out of 2934 seeds). Regarding sites, germination was 0.92 % in Manta and 0.56 % in Castrovirreyra.

In the greenhouse, *P. flavipila* seeds exhibited epigeal germination, starting at 33 days and ending at 69 days, with a germination rate of 0.64 ± 1.11 % (14 germinated seeds). The mean germination rates per site were 1.29 % and 0.76 % for Manta and Castrovirreyra, respectively.

In both tests, the germinated seeds had a greater mass (5.31 ± 1.40 mg and 6.28 ± 1.94 mg for standard and greenhouse germination, respectively) than those that did not germinate (3.44 ± 1.72 mg and 3.52 ± 1.74 mg for standard and greenhouse germination, respectively).

4. Discussion

The results demonstrate that seed mass and maternal characteristics are significantly associated with the viability of *Polylepis flavipila* seeds. Seed mass exhibited high variability both within and between mother trees and sites. Overall, physiological seed quality, measured through viability, standard germination, and greenhouse germination, was very low. In agreement with our hypothesis H_1 , seed mass was strongly associated with viability and emerged as the primary explanatory factor. Regarding hypothesis H_2 , maternal tree height was the only maternal characteristic positively associated with seed viability. Furthermore, in accordance with our hypothesis H_3 , the low seedling number was directly correlated with seed viability. These findings offer valuable

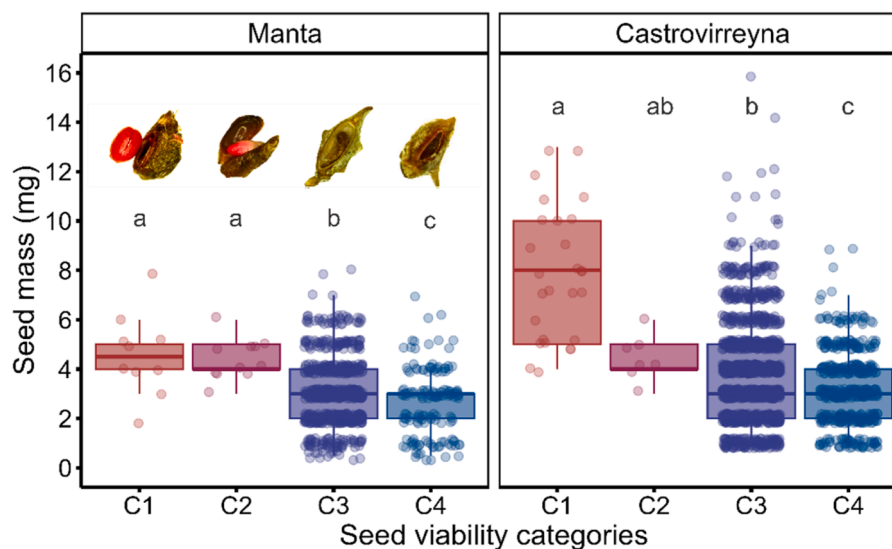


Fig. 4. Boxplot of seed mass for each viability category. The vertical lines represent $\pm 1SD$ and the letters above the bars show the results of the Multiple Comparisons of Means - Tukey Contrasts. Different letters indicate statistically significant differences between stages ($p < 0.01$). C1: Viable seed. C2: Viable seed with a proportion of non-viable tissue. C3: Non-viable seed – seed degraded or dead embryo tissue. C4: Non-viable seed – seed without embryo tissue.

Table 2

Results of the binomial GLMM (link=logit) of *Polylepis flavipila* seed viability in relation to seed mass, site and mass × site interaction.

	Parameter estimates	Estimate	Standard error	CI 95 %	Z value	Pr(> z)
Fixed effects	Intercept	-4.12	0.49	[-5.07, -3.16]	-8.42	<0.001
	Seed mass	1.86	0.53	[0.82, 2.89]	3.51	<0.001
	Castrovirreyña	-1.06	0.66	[-2.35, 0.23]	-1.6	0.11
	Seed mass × Castrovirreyña	-0.41	0.67	[-1.72, 0.90]	-0.61	0.54
	Site:Tree	Variance	Standard deviation	Correlation		
Random effects	Random Intercept	0.96	0.98	0.55		
	Random slope	1.03	1.01			

In the fixed effects: Intercept represents the parameter estimate of the probability that a seed of mean mass is viable (logit scale) in Manta. Castrovirreyña, shows the differential of intercepts between Castrovirreyña and Manta sites. Seed mass, shows the estimated slope (logit scale) or rate of change per 1 SD increase in seed mass for Manta. Seed mass × Castrovirreyña, denotes a differential of slopes between Castrovirreyña and Manta.

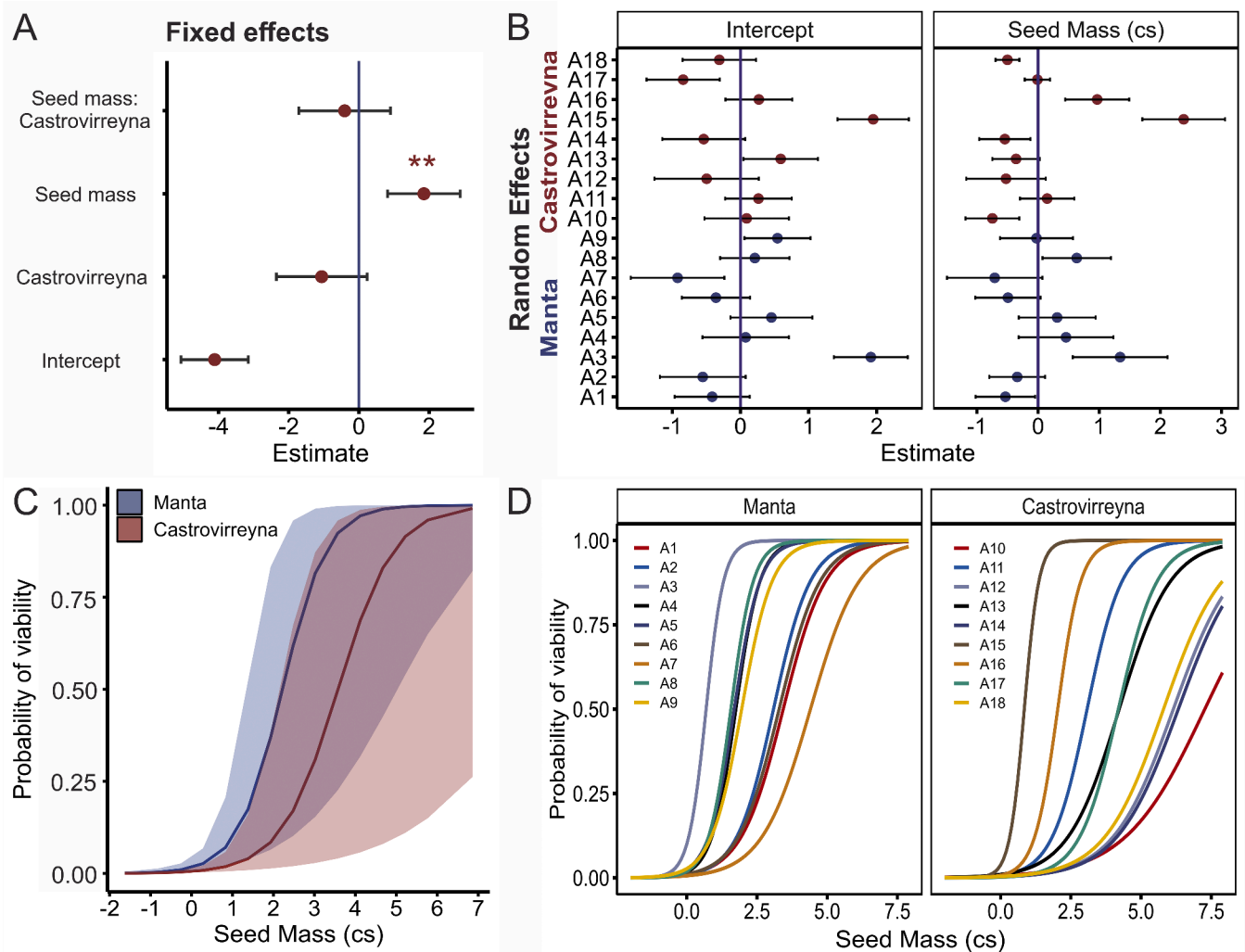


Fig. 5. Plot of the estimators and conditional curves predicted by the model. A Parameter estimates and 95 % confidence intervals of the fixed effects (only the variable seed mass was significant). B Random intercept and slope coefficients, and their standard errors, for each evaluated tree nested in the sites (A1-A18 are the evaluated trees). C Model predictions at the population level in relation to mass (cs) and site (shaded areas indicate 95 % CI). D Model predictions at the group level for each tree and site (each color represents a different tree). Seed mass is centered and standardized: mean = 3.49 and SD = 1.76.

information to improve seed collection strategies: trees 4 m or taller and seeds 5 mg or larger can achieve a mean seed viability of 15.94 %. Therefore, the selection of seeds with these characteristics ensures acceptable levels of seed quality for seedling production. In addition, in the field, a greater number of seedlings under the tree crown is associated with seeds with greater viability.

4.1. *Polylepis flavipila* seed mass

Polylepis flavipila seed mass showed considerable variability both within and between trees and between sites, a pattern consistent with observations in other species of the genus. For example, in *P. australis*, the average seed mass (4.6 mg, range: 2–14.7 mg) showed significant variations between geographic regions or forest fragments, and within a

Table 3

Results of the multi-model inference approach. The best fitted models show the relationship between random coefficients (intercept and slope) and maternal characteristics and maternal environment.

Random coefficients	Model	Estimate	Standard error	Pr(> t)	AICc	R ²
Random intercept	Intercept	-0.038	0.013	0.01	-92.46	0.54
	Height	0.013	0.003	<		
	total			0.001		
Random slope	Intercept	0.823	0.022	<0.001	-30.62	0

The best model explaining the random slope was null.

single tree (Seltmann et al., 2007). Similarly, *P. tomentella* (5–18 mg) and *P. incana* (3–30 mg) also exhibit wide variations in seed mass (Domic et al., 2020; Quispe-Melgar et al., 2024a). These differences suggest that variability in seed mass could be associated with maternal characteristics, the maternal environment or the interaction between both factors. We found statistically significant differences between seed mass from Manta and Castrovirreyna, highlighting the influence of micro- and macro-scale environmental factors on seed traits and the potential role of those factors in local adaptation.

The high variability observed in *P. flavipila* may reflect both landscape heterogeneity and small-scale environmental variations characteristic of *Polylepis* forests (Cingolani et al., 2004). These shared conditions in high-altitude environments influence seed development by affecting resource allocation in maternal trees. For example, associations have been reported between seed mass and factors, such as the type of surrounding vegetation cover in *P. subtusalbida* (Gareca et al., 2018); elevation in *P. australis* (Marcora et al., 2008) and characteristics of the maternal tree, such as height in *P. australis* and *P. tomentella* (Domic et al., 2020; Pollice et al., 2013). These findings suggest that the wide variability in seed mass could be an evolutionary adaptation to the heterogeneous high-altitude environment or a characteristic trait of the genus *Polylepis*.

4.2. Seed viability, standard germination and greenhouse germination

The low viability and germination rate of *P. flavipila* seeds observed in this study is attributed to the absence of embryonic tissues or their predominance at both stages of degradation. Regarding the four viability categories, we do not rule out the possibility that there is a degradation gradient; thus, C1 seeds (viable) can become C2 (partially viable) and these in turn can become C3 (degraded) or C4 (empty) if the degradation process continues. However, to test this hypothesis, further research is needed to explore the entire ontogenetic development of fruits and seeds in *P. flavipila*.

Likewise, it is also likely that the seeds do not follow this degradation pattern; thus, for example, C4 seeds (empty) may have never had an embryo or the embryo may have been aborted shortly after fertilization. Previous studies in other *Polylepis* species attribute low seed viability to different factors: (1) self-incompatibility of prezygotic or early postzygotic seeds and biparental inbreeding depression (Domic et al., 2017; López et al., 2021; Seltmann et al., 2007); (2) potential floral fungal infection (Domic et al., 2017); (3) parthenocarpy or resource limitation due to forest degradation, particularly of soil (Renison et al., 2004); and (4) pollen limitation due to forest fragmentation (López et al., 2021). Viability rates in *Polylepis* species generally range from 15 % to 58 %, with the lowest values reported for *P. pacensis* (15 %) and the highest for *P. neglecta* (58 %). Germination rates vary between 2 % (*P. incarum*) and 31 % (*P. incana*) (Cuyckens et al., 2021; Gareca et al., 2012; Quispe-Melgar et al., 2024a; Seltmann et al., 2007; Vega et al., 2018). In this context, *P. flavipila* likely has the lowest seed viability and germination rates reported for the genus.

Seed mass played a significant role in seed viability, since larger seeds were generally found to be more viable, as previously reported for

other *Polylepis* species (Domic et al., 2020; Quispe-Melgar et al., 2024a; Seltmann et al., 2007). However, the presence of non-viable seeds across all mass ranges raises questions about the reasons why trees produce very small seeds (≤ 1 mg), why heavier seeds are typically more viable, and why some of the heaviest seeds are non-viable. Longitudinal studies analyzing seed development from fertilization to maturation could provide insights into these patterns.

We recorded the presence of two or more seeds within a single fruit, especially in seeds with high mass, a phenomenon not previously reported for the genus. *Polylepis* is considered to produce flowers with a single ovule and, therefore, a single embryo and a single seed (López et al., 2018). Hence, these results suggest that some flowers may produce more than one ovule or, alternatively, that the same ovule can generate more than one embryo, a process known as polyembryony, a type of apomictic reproduction (Batygina and Vinogradova, 2007; Lakshmanan and Ambegaokar, 1984). This finding raises new questions and research opportunities on the reproductive biology of seeds of the genus *Polylepis*.

4.3. Maternal characteristics, maternal environment and seed viability

Modeling mother trees as a random effect increased the percentage of variability explained by the model, supporting the statistical approach used in this study. In the secondary analysis, we observed that mother tree height is associated with the predicted probability of seed viability for each tree. These findings are in line with those of Domic et al. (2020), who reported a positive association between *P. tomentella* tree height and seed physiological quality. Additionally, we did not find a significant association between viability and ground-level diameter or crown diameter, although these maternal characteristics were strongly correlated with tree height and number of seedlings ($r > 0.7$).

We found that seed viability can explain about 25 % of the variation in the number of seedlings ($r = 0.56$). Moreover, given the low seed viability observed in *P. flavipila*, it is likely that this factor plays a key role in explaining, at least in part, the number of seedlings under the mother tree. However, this would not be a cause-and-effect relationship but an association, since there are other factors, such as the availability of suitable microsites for germination, environmental conditions or even seed productivity, which also influence the number of seedlings under the mother tree. Therefore, although indirectly and partially, a higher number of seedlings under the tree canopy would be associated with trees with better quality seeds.

5. Challenges and strategies for restoration and conservation

Ongoing efforts for high-Andean forest restoration involve the planting of millions of saplings of several *Polylepis* species (<https://www.globalforestgeneration.org/>) and it would be desirable to include *P. flavipila* in these projects. However, the low viability and germination of its seeds pose a great challenge to direct seeding or sapling production-based restoration initiatives. Here we suggest that criteria such as tree height or the presence of natural regeneration under the tree crown can be used as indicators of seed quality to balance genetic and physiological attributes and thus facilitate restoration efforts.

We found degraded seeds of various sizes and at different stages, suggesting the presence of a degrading agent. Therefore, the collected seeds should be used immediately or subjected to disinfection and controlled storage. During selection, very small seeds may be discarded, but it is important to avoid using only large seeds to preserve genetic diversity. Additionally, given the limited number of viable seeds, it is advisable to complement restoration efforts with natural regeneration. Reforestation aimed at soil protection or water recharge should include strategies for the collection and asexual propagation of *P. flavipila* to maximize the production of plants with adequate genetic diversity. Finally, given the difficulties in *P. flavipila* propagation, the conservation of existing forests is especially important, as already mentioned by

Ames-Martínez et al. (2021). To this end, education and awareness raising among local communities play a fundamental role.

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors did not use any AI or AI-assisted technologies.

CRediT authorship contribution statement

L. David Huayta-Hinojosa: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Harold Rusbelth Quispe-Melgar:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Conceptualization. **Katherine Lucero Lagones Poma:** Writing – review & editing, Investigation. **Yashira Stefani Llacua-Tineo:** Writing – review & editing, Investigation. **Fressia Nathalie Ames-Martínez:** Writing – review & editing, Resources, Investigation. **Daniel Renison:** Writing – review & editing, Supervision, Methodology.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Harold Rusbelth Quispe Melgar reports financial support was provided by National Fund For Scientific Technological and Technological Innovation Development. The authors declare no conflicts of interest. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The database that supports this study is available at <http://hdl.handle.net/11336/244072>

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